At page 4, line 10 (line 8 in the unexecuted copy), "tmeperature" has been replaced with —temperature—

At page 4, line 16 (line 14 in the unexecuted copy), "glyucine" has been replaced with —glycine—

At page 4, line 18 (line 16 in the unexecuted copy), "prepration" has been replaced with —preparation—

At page 4, line 28 (line 26 in the unexecuted copy), "prepartion" has been replaced with —preparation—

As discussed in the response, mailed February 17, 2000, the DECLARATION of Perez describes the generation of transgenic mice using methods and materials disclosed in the above-referenced application, which describes in detail each of the processes involved in the generation of transgenic animals using artificial chromosomes, and standard methods as described in the DECLARATION. The DECLARATION of Perez demonstrates that using methods described in the application and standard methods of DNA manipulation, it was possible to generate transgenic mice employing artificial chromosomes as disclosed in the application. Transgenic mice were produced by microinjection of 60 Mb murine satellite DNA-based artificial chromosomes containing multiple copies of the *lacZ* (β-galactosidase) and *hph* (hygromycin phosphotransferase) genes into the pronucleus of mouse zygotes.

Successive steps in the process of transgenic mice production were analyzed, including the presence and expression of artificial chromosomes in preimplantation embryos and the presence and integrity of the artificial chromosomes in founder and progeny mice, and the results are presented in the DECLARATION. As described in the DECLARATION, fluorescence *in situ* hybridization (FISH) analysis of preimplantation embryos injected with satellite DNA-based artificial chromosomes demonstrated that 44% of the analyzed embryos contained intact satellite DNA-based artificial chromosomes in 8-67% of the total cells analyzed for each positive embryo. In β-galactosidase staining assays of injected preimplantation embryos at various developmental stages, 31% of the analyzed embryos showed X-gal staining indicating the presence of a functional marker gene in the artificial chromosomes that was expressed in a mosaic pattern.

Seven percent of the mice born after implantation of injected embryos into pseudopregnant female mice were positive for the *hph* gene in nucleic acid amplification analyses of tail DNA, and mating of a transgenic female founder with wild-type F1 males yielded progeny, 46% of which were positive for the presence of the *hph* gene in nucleic acid amplification assays of tail DNA. FISH analysis of mitogen-activated peripheral blood lymphocytes from the female transgenic founder revealed the presence of intact, discrete satellite DNA-based artificial chromosomes, which had not integrated into the endogenous chromosomes, in approximately 60% of the cells. FISH analysis of peripheral blood lymphocytes from progeny carrying the satellite DNA-based artificial chromosomes revealed that intact artificial chromosomes were present in approximately 60% of the analyzed cells with no apparent translocation of the artificial chromosome DNA onto the host chromosomes.

The results of these analyses demonstrate that satellite DNA-based artificial chromosomes as described in the above-referenced application can be used in standard methods of transgenic animal generation to yield viable transgenic animals containing within their cells intact, discrete, heterologous gene-containing artificial chromosomes as autonomous, stably replicating, extra-genomic elements. Furthermore, the results of these analyses demonstrate that the satellite DNA-based artificial chromosomes are transmitted through the germline.

In view of the above remarks and the amendments and remarks of record, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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